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Reactions were overlaid with sterile mineral oil and carried out in a thermocycler (Perkin-Elmer DNA Thermal Cycler 480). Thermocycling parameters employed after at 96°C, denaturation for 10 min were 28 cycles of 1 min at 94°C, 1 min at an annealing temperature, and 2 min at 72°C. A further extension step involving 1 min at 48°C and 5 min at 72°C was also employed. The primers used were 27f (5'-AGAGTTGATCCTGGCTCAG-3') (SEQ ID No:8) and 1492r (5'-GGTTACCTTGTACGACT-3') (SEQ ID No:9) (Lane 1991). In some cases GeneReleaser (Bioventures Inc., Tennessee, USA) was used according to the manufacturer's instructions in the reactions outlined above. PCR products were purified using a QIAquick PCR purification kit (QIAGEN, Victoria Australia) according to the manufacturer's instructions.

Sequencing of 16S rDNA

All 16S rDNA samples were initially partially sequenced using the universal 16S rRNA primer, 530f (5'-GTGCCAGCMGCCGCGG-3') (SEQ ID. No: 10) and an ABI Big Dye Terminator Cycle Sequencing Ready Reaction Mix kit (Victoria, Australia). Selected 16S rDNA were subsequently fully sequenced on both strands using the following primers: 519r (5'-GWATTACCGCGGCKGCTG-3') (SEQ ID No:11), 27f, 907r (5'-CCGTCAATTCTTTAGTTT-3') (SEQ ID No:12), 926f (5'-AAACTYAAAKGAATTGACGG-3') (SEQ ID No:13), and 1492r (Lane, 1991).

Approximately 100 ng of purified PCR product and 25 ng of primer were used in the sequencing reactions. Thermal cycling was carried out in an MJ Research PTC-100 thermocycler with an initial denaturation step of 96°C for 2 min, following by 25 cycles of 50°C for 15s, 60°C for 4 min, and 96°C for 30s. The resulting cycle sequencing products were purified using the ethanol plus sodium acetate method (ABI, Australia). Purified sequencing products were submitted to the Australian Genome Research Facility for analysis on an Applied Biosystems 377 automated sequencer.

Phylogenetic analysis

Phylogenetic analysis of 16S rDNA's was carried out according to Dojka et al. (1998). Briefly, sequences were aligned and compiled in SeqEd (Applied Biosystems Australia). Compiled sequences were compared with those on publicly available databases by use of the BLAST (Basic Alignment Search Tool) (Altschul et al. 1990) to determine approximate phylogenetic affiliations. Compiled sequences were then aligned using the ARB software package (Strunk et al. unpublished) and refined manually. Phylogenetic trees based on comparative analysis of the 16S rRNA genes were construction by performing evolution distance analyses on these alignments using the

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